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# QUANTITATIVE CHANGES IN HORMONE RECEPTORS EXPRESSION LEVEL IN BREAST CANCER PATIENTS. A RETROSPECTIVE COHORT STUDY

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## Abstract

Although hormone receptors discordance in the evolution of breast cancer was extensively studied, it almost always has been treated as a dichotomous variable, disregarding their absolute values. The degree, the direction and the significance of quantitative variations in time in the level of expression of estrogen receptors (ER) and progesterone receptors (PR) are largely unknown.

We performed a retrospective analysis of quantitative changes in the level of ER and PR in paired samples from either primary or recurrent lesion from the same patient in two separated points in time. ER and d PR expression was recorded as the percentage of staining cells. Subgroups analyses were not pre-planned.

Sixty-eight females with breast cancer of any stage were included. Wilcoxon signed-rank test indicated a statistically significant reduction in the ER expression between first and second ER determination (Z=-2.75, r=-0.23, p=0.006). For PR, the difference was not statistically significant. In the subgroup analyses, after Bonferroni correction, only exposure to endocrine treatment, tissue obtained by surgery and age >40 years were significantly associated with the decrease in the ER expression level.

Even though random error and technical issues are likely the main sources of the ER variability, the results of our study suggest a trend to a decrease in ER expression, in the relationship with tissue sampling methodology and/or exposure to endocrine therapy.

**Key words:** estrogen receptors, hormone receptors, quantitative variation, endocrine therapy, tissue sampling method

## Resumen

Si bien se estudió ampliamente la discordancia de los receptores hormonales en la evolución del cáncer de mama, casi siempre se trató como una variable dicotómica, sin tener en cuenta sus valores absolutos. El grado, la dirección y la importancia de las variaciones cuantitativas en el tiempo en el nivel de expresión de los receptores de estrógeno (RE) y los receptores de progesterona (RP) son en gran parte desconocidos.

Realizamos un análisis retrospectivo de los cambios cuantitativos en el nivel de RE y RP en muestras pareadas de lesiones primarias o recurrentes del mismo paciente en dos puntos separados en el tiempo. La expresión de RE y d RP se registró como el porcentaje de células teñidas. Los análisis de subgrupos no fueron planificados previamente.

Se incluyeron 68 mujeres con cáncer de mama de cualquier estadio. La prueba de rango con signo de Wilcoxon indicó una reducción estadísticamente significativa en la expresión de RE entre la primera y la segunda determinación de RE (Z=-2.75, r=-0.23, p=0.006). Para RP, la diferencia no fue estadísticamente significativa. En los análisis de subgrupos, después de la corrección de Bonferroni, sólo la exposición al tratamiento endocrino, el tejido obtenido mediante cirugía y la edad >40 años se asociaron significativamente con la disminución en el nivel de expresión de RE.

A pesar de que el error aleatorio y los problemas técnicos son probablemente las principales fuentes de la variabilidad de la RE, los resultados de nuestro estudio sugieren una tendencia a una disminución en la expresión de la RE, en la relación con la metodología de muestreo de tejidos y/o la exposición a la terapia endocrina.

**Palabras clave:** receptores de estrógeno, receptores de hormonas, variación cuantitativa, terapia endocrina, método de muestreo de tejido

## Introduction

Biomarker status determination plays an essential role in clinical decision-making in patients with invasive breast cancer. Estrogen receptors (ER) and progesterone receptors (PR) measurement are mandatory at the time of the initial diagnosis and it is recommended, when feasible, in recurrent lesions[1,2]. Several studies investigated the discordance in ER and PR expression between immunohistochemically (IHC) assessed tissue from primary tumors and paired second primary, loco-regional and metastatic recurrences with dissimilar results[3-7]. In a meta-analysis of 39 studies, published by Schrijver *et al,* after the mean follow-up of 51 months, pooled estimates of ER conversion rates from positive to negative and vice versa both were around 22%. Pooled estimates of PR conversion rates from positive to negative were 49% and from negative to positive 16%, making apparent a trend toward lesser hormone receptors expression[8].

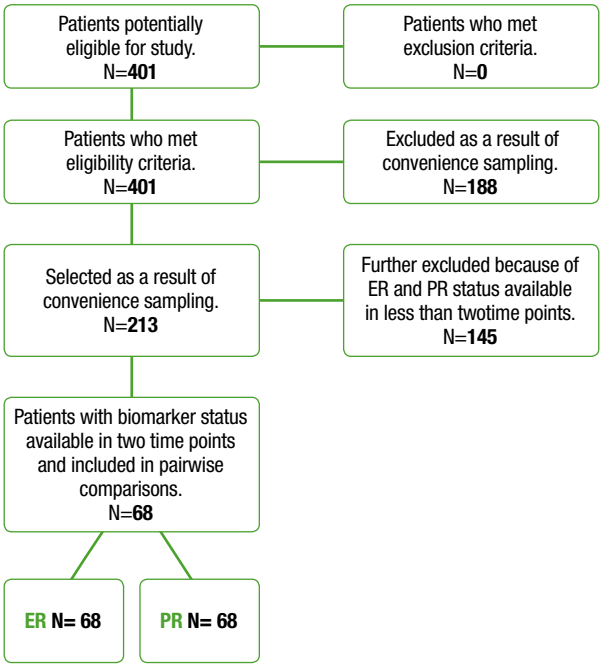
However, all of these studies treated hormone receptors expression as a binary variable (positive/negative) providing few insights, if any, into the relation of the phenomenon with the intrinsic tumor characteristics or extrinsic environmental factors, such as treatment exposure. We are not aware of any relevant study addressing quantitative changes in hormone receptors expression in a comprehensive manner. Thus, although in the study by Dieci *et al,* reporting on ER and PR conversion rates, quantitative absolute changes in ER and PR were displayed as waterfall plots, data analyses were not provided7. The lack of interest in ER and PR expression as a quantitative continuous variable is likely due to issues of assay reproducibility and lack of the uncontroversial demonstration of a strong relationship between quantitative hormone receptor expression and clinically important outcomes[9,10]. Nevertheless, as more protracted adjuvant endocrine treatments became more utilized and new drugs for advanced hormone receptor-positive breast cancer more universally available, the practical relevance of the quantitative hormone expression might increase. Also, the study of quantitative variations in ER and PR may contribute to a better understanding of the time behavior of the neoplastic process.

We aimed to study quantitative changes in ER and PR expression in paired samples corresponding to two separate points in time and their relationship with some clinical features and treatment exposure.

## Materials and methods

We performed a retrospective pairwise comparison of the level of ER and PR expression in breast cancer. Patients have been retrieved from a prospectively acquired database in the Department of Pathology at Hospital Dr. Juan A. Fernández. We included females of any age with the histopathologic diagnosis of invasive breast cancer admitted between October 10th, 2010 and October 17th, 2017. Metastases to the breast, mesenchymal and lymphoid tumors were the exclusion criteria. Selected patients were further screened for the availability of quantitative data on the level of expression of ER and PR, either on biopsies or surgical specimens of primary tumor or metastases in two separated time points. The cut-point between synchronous and metachronous lesions has been set in six months. Initially, we had planned to screen all eligible patients. However, recruitment was prematurely closed after 213 patients have been included by a non-probabilistic convenience sampling. The study selection process is shown in Figure 1.

**Figure 1.** Flow chart of the study selection process




From non-electronic clinical charts of selected patients, we extracted data on ER and PR status and treatment exposure. Information on primary antibody and IHC assay protocol was absent in almost all clinical charts and was not recorded.

Hormone receptor expression was treated as a continuous variable and recorded as a percentage of staining cell. The main outcome was quantitative changes in ER expression in percentage points between two time points: ΔER=ER2-ER1, ΔPR=PR2-PR1. Continuous variables were not normally distributed and data was paired. Thus, to test against the null hypothesis that there is no difference between the percentage of staining cells in two time points we performed a two-tailed Wilcoxon signed-rank test. Subgroup analyses were not pre-planned. Bonferroni correction for multiple comparisons was applied, except for those cases in which p-values could not be accurately calculated because of the small N. As an effect size measure we used r statistic ($r=Z/\sqrt{N}$)[11]. The effect size was graded as small if r was between 0.10 and 0.39, medium if r was in the range 0.40-0.59, and large if r value was 0.60 or higher, as it was suggested by Mangiafico11. Spearman´s rank correlation was used for strength of association measurements. The degree of correlation was interpreted following the guidance provided by Mukaka[12]. Data on time period between hormone receptor analyses was log-transformed, but continued to follow a non-normal distribution. Thus, the Kruskal-Wallis test was performed to examine the relationship between ΔER and ΔRP and time interval. All tests were done at alpha level 0.05.

For grouping purposes, we defined as a high ER o PR expression levels between 81% and 100%, intermediate - those between 21 and 80%, low-between 1% and 20%, and null-0%. Based on this classification we put forward a model of the ER transition between groups. Simple descriptive statistics were performed in Microsoft Excel©. For Wilcoxon signed-ranked test and Kruskal-Wallis test we used the online tool Social Science Calculator[13].

## Results

Patient selection process is shown in Figure 1. From 401 eligible patients, 213 were included by a convenience sampling. Of them, after the *screening* for pairwise pathology reports availability, we selected 68. The mean age was 51.7 (SD: +/-14.4) years. The initial stage was I in 10.5%, II in 34.3%, III in 50.7%, and IV in 4.5%.

The tissue samples for the first pathological exam were obtained from the primary tumor in 98.5% and a systemic metastasis in 1.5%. It was obtained by biopsy in 77.9% and came from surgery in 22.1%. The tissue samples for the second pathological study came from the same primary in 80.3%, synchronous contralateral primary in 4.6%, local recurrence/metachronous ipsilateral primary in 6.1%, metachronous contralateral primary in 1.5%, ipsilateral regional lymph nodes in 1.5%, synchronous or metachronous distant metastases in 6.1%. It was obtained by biopsy in 4.5% and by surgery in 95.5%, (Table 1). The median time between the first and second pathology report was 0.55 years, interquartile range (IQR): 0.14-0.98 years.

**Table 1.** Sampled lesions and sampling methods

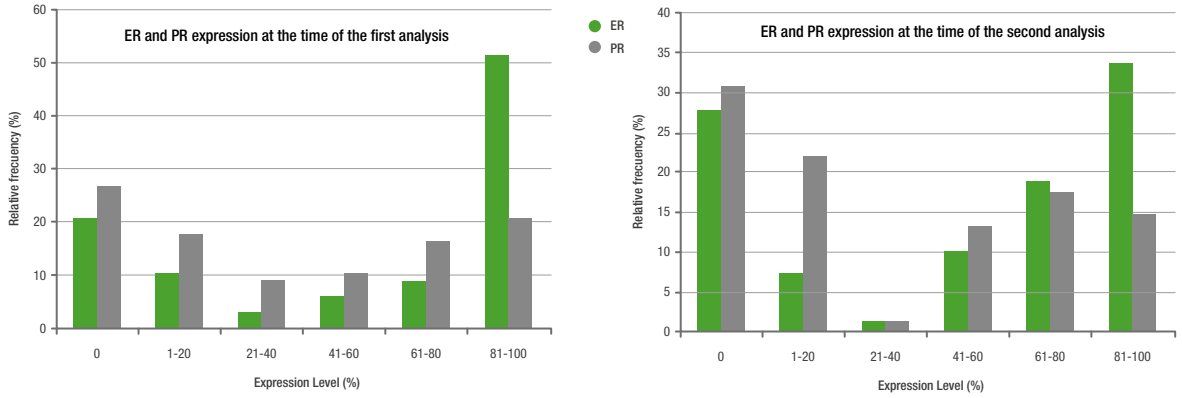
| | First analysis | Second analysis |
|---|---|---|
| | **Sampled lesion** | |
| Primary tumor | 98.5% | 80.3% |
| Synchronous contralateral primary tumor | - | 4.6% |
| Metachronous ipsilateral primary tumor/local recurrence | - | 6.1% |
| Metachronous contralateral lesion | - | 1.5% |
| Ipsilateral regional lymph nodes recurrence | - | 1.5% |
| Systemic metastasis, synchronous or metachronous | 1.5% | 6.1% |
| | **Sampling method** | |
| Biopsy | 22.1% | 4.5% |
| Surgery | 77.9% | 95.5% |

Between the first and second hormone receptor analysis participants received the following treatments: chemotherapy 49.2%, endocrine therapy 15.4%, both 6.2%, none 41.5%. Chemotherapy regimens included anthracyclines, taxanes, their combination or CMF. Endocrine therapy comprised tamoxifen or aromatase inhibitors with or without gonadotropin-releasing hormone analogs. No patient was exposed to palbociclib.

At the baseline, the median of ER was 85% (IQR: 5.8 -90.0%)), the median of PR was 40% (IQR: 0.0-80.0%). The distribution according to the levels of ER and PR expression at the time of the first and the second analyses is shown in Figure 2. At the baseline, more than half of patients had ER in the range between 81% and 100%; 21% of patients had a negative status of ER.

The main results of the comparisons are summarized in Table 2. In a two-tailed Wilcoxon signed-rank test ER expression at baseline were significantly higher than at the time of the second analysis (Z=-2.75, r=-0.23, p=0.006).

**Figure 2.** Quantitative estrogen receptors and progesterone receptors expression at the time of the first and second analysis




*ER: estrogen receptors; PR: progesterone receptors*

**Table 2.** Quantitative variations in estrogen receptors and progesterone receptors level. Subgroup analysis

| | | | | Wilcoxon signed-rank test | | | After the Bonferroni correction (p<0.027) |
|---|---|---|---|---|---|---|---|
| Subgroup | | | N | Z-value | p-value | r | |
| ER all | | | 68 | -2.75 | 0.006 | -0.23 | NA |
| PR all | | | 68 | -1.85 | 0.064 | -0.16 | - |
| ER Chemo | | yes | 32 | -1.64 | 0.101 | -0.21 | - |
| | | no | 33 | -1.85 | 0.064 | -0.23 | - |
| PR Chemo | | yes | 32 | -2.02 | 0.043 | -0.25 | |
| | | no | 33 | -0.23 | 0.818 | -0.03 | NS |
| | | | | | | | - |
| ER Endocrine | | yes | 10 | -2.36 | <0.05* | -0.53 | NA* |
| | | no | 55 | -1.24 | 0.215 | -0.12 | - |
| PR Endocrine | | yes | 10 | -0.98 | >0.05 | -0.22 | - |
| | | no | 55 | -1.49 | 0.136 | -0.14 | - |
| ER | Age | ≤40 | 15 | -0.52 | >0.05 | -0.09 | - |
| | | >40 | 42 | -3.67 | 0.0002 | -0.40 | S |
| | | ≤40 | 15 | -0.98 | >0.05* | -0.25 | - |
| PR | Age | >40 | 42 | -1.76 | 0.08 | -0.19 | |
| Tissue for the first analysis obtained by** | Biopsy | ER | 51 | -1.93 | 0.054 | -0.19 | - |
| | | PR | 51 | -1.44 | 0.150 | -0.14 | - |
| | Surgery | ER | 15 | -2.40 | 0.016 | -0.44 | S |
| | | PR | 15 | -1.45 | 0.147 | -0.26 | - |

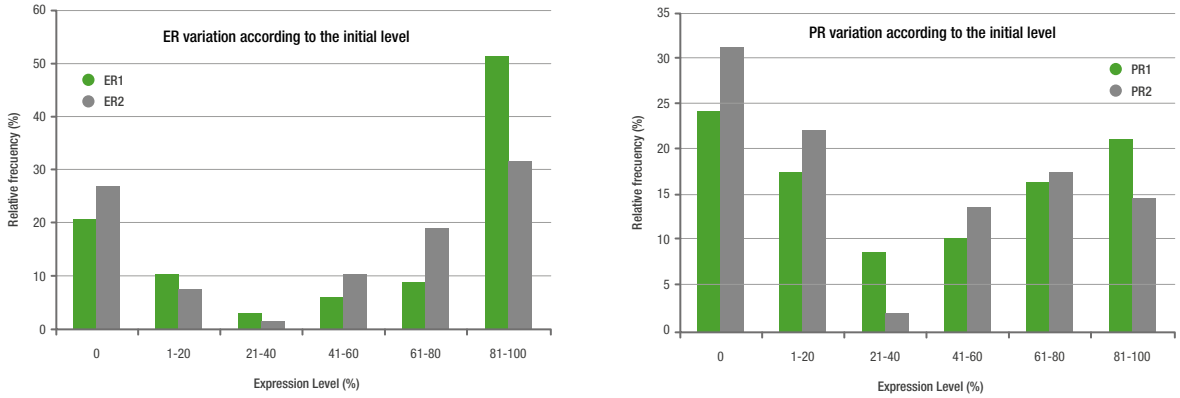
*ER: Estrogen receptors; PR: Progesterone receptors; S: Significant; NS: Not significant; NA: Not applicable*
*N is not large enough for the distribution of the Wilcoxon W statistic to form a normal distribution. Therefore, it is not possible to calculate accurate p-value13
**Data available for 66 patients

For PR a similar trend was observed, but it was not statistically significant (Z=-1.85, r=-0.16, p=0.064).

Variations in ER and PR expression according to their initial level are shown in Figure 3.

In subgroup analyses, age older than 40 years, endocrine therapy and tissue obtained by surgery were significantly associated with a decrease in the expression of ER, while chemotherapy with a reduction of PR (Table 1). When Bonferroni correction has been applied, the association between chemotherapy and variation in PR level was no longer statistically significant. After the exclusion of contralateral tumors, results have not been changed (not shown). No significant difference in the expression of ER at baseline between samples obtained by biopsy (median 80%) or surgery (median 90%) was observed in two-tailed Mann-Whitney U-test, (U=354.5, p= .50). Similar results were found for PR, (median 30% and 60% respectively, U=303.5, p=0.23).

**Figure 3.** Variations in estrogen receptors and progesterone receptors according to the initial level of expression

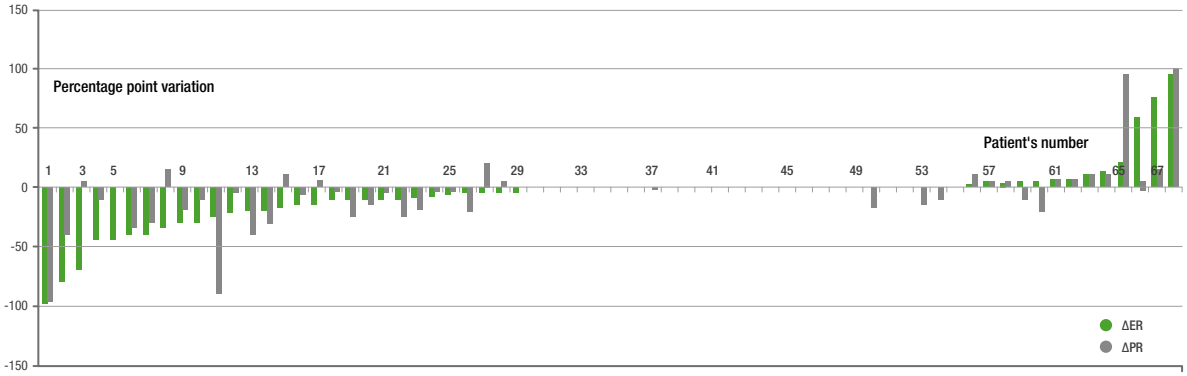



*ER: estrogen receptors; PR: progesterone receptors*

We have examined the relationship between ΔER and the time interval between analyses. When the time interval between matched samples was less than 0.1 years, the median ΔER was equal to 0 percentage points. When it was between 0.1 and 1.0 years, median ΔER was -1 percentage points, and when it was more than 1.0 years, median ΔER was -13 percentage points. However, the observed trend was not statistically significant in the Kruskal-Wallis test, (H=2.67, p=0.26). Similar results were obtained for PR variation, (not shown).

A very strong positive correlation between ER and PR expression level at baseline was observed in two-tailed Spearman´s rank correlation test (rs=0.94, p=0.0048). The degree of correlation between ER and PR at the time of the second analysis, between ER1 and ER2, and between PR1 and PR2 was lesser but still high (rs=0.83, p=0.042). Correlation between ΔER and ΔPR is shown in Figure 4.

**Figure 4.** Correlation between ΔER and ΔPR

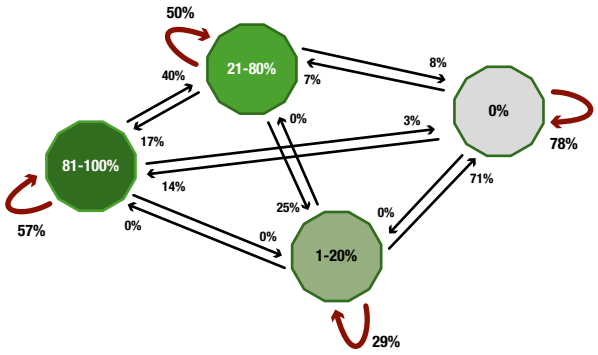



Finally, we have studied probabilities of transition between states of high, intermediate, low and null ER expression. Results are displayed as a graph in Figure 5. Remaining in the same group was the most probable scenario in all classes except for the low expression group. From this group, no transition to a higher expression class was observed. High expression group almost always conserved high or intermediate level of expression. In the null expression group, staying in the same class was, by far, the most common scenario, although the transition to a high expression class infrequently has occurred.

## Discussion

We performed a retrospective analysis of quantitative variations in hormone receptor expression. A statistically significant, small size decrease in ER expression was detected. For PR, trends similar to those observed for ER have been noticed, but they were not statistically significant.

**Figura 5.** Probabilities of transition between states of high, intermediate, low and null estrogen receptors expression




*ER: Estrogen receptors*
Nodes are states of high (81%-100%), intermediate (21%-80%), low (1%-20%) and null (0%) ER expression. Edges (straight black arrows) stand for probabilities of transition between states. Red curved arrows stand for probabilities of remaining in the same state.

No statistically significant relationship between hormone receptor changes and time interval was found.

Age older than 40 years, exposure to endocrine therapy and tissue for the first analysis obtained from a surgical specimen were significantly associated with a decrease in ER expression in the univariate analysis. Multivariate analysis was not conducted, because the number of patients was too small in the subgroups of interest. However, in the subgroup of patients younger than 41 years, only 7% underwent endocrine therapy and only in 13% the tissue for the first analysis came from surgery, whereas in the subgroup of the older patients the respective percentages were near twice as high. All this make us suppose that age may not be independently related to the changes in the ER expression level.

The tissue sampling method used may influence the results of hormone receptors determination. In the study by Chen at al, although overall concordance was high, core needle biopsy specimens were associated with higher positive ER and PR rates (2.2% and 3.3% respectively) when compared to open excisional biopsy. Putative reasons invoked to explain the phenomenon were delayed fixation, under-fixation/over-fixation before IHC and more intense staining in the periphery of the tumor than in the center, but also random sampling error and tumor heterogeneity14. Additionally, it can be hypothesized that tissue trauma and ischemia, coupled with a surgical procedure, may downregulate hormone receptor expression. Recently, Gao *et al* reported a significant impact of the sampling method on the regulation of gene expression in ER-positive breast cancer. However, we failed to identify ESR1/PGR in the list of the top-ranked up- and downregulated genes provided by authors15. Even if the aforementioned considerations are taking into account, the exposure to endocrine therapy would not be an implausible explanation for the decrease in the level of ER, albeit the number of patients is small. In line with this, ESR1/PGR mRNA expression downregulation in metastatic lesion compared with primary tumors has been reported16. The clinical significance of the described changes is unknown.

In our study, patients with an absolute ER expression of 0% had the lowest probability of transition to other states. This fact, in combination with a general trend towards a lower ER expression, would enable us to put forward a hypothesis that the null ER expression is a more stable state than others.

A possible interpretation could be that all observed changes in ER expression are a result of the lack of the assay reproducibility (random error). However, two arguments can be opposed to this reasoning. First, the difference in ER expression between two time points was statistically significant. Second, if the observed differences were purely artefactual, similar probabilities of transition between groups of higher and lower ER expression would be expected, whereas, in fact, a sizable difference was observed, despite no statistical analyses of these data were performed. Shighoko *et al*17, treating ER expression as a binary variable and using a Bayesian misclassification correction method found that "technical misclassification accounted for 53%–83% of the ER discordance between synchronous primary cancers and 11%–25% of ER discordance between metachronous cancers". Also, positive-to-negative changes were four times more frequent, than negative-to-positive in the study by Shighoko *et al*17.

We want to stress, that the findings of our study are of a hypothesis-generating nature and need to be validated in an independent and larger set of observations. Ideally, it would be a prospective study of a sufficient statistical power, which uses a uniform, standardized assay methodology.

Our study has several limitations. The non-probabilistic convenience sampling method used and the high proportion of eligible patients excluded make it prone to the selection bias.

Sample size estimation was not performed and the number of patients included was small for many analyses. As the primary antibody and other relevant elements of the assay were not recorded, information bias cannot be discarded.

To our knowledge, this is the first study which quantitatively assesses changes in the absolute hormone receptors expression and attempts to relate them to the treatment exposure.

In conclusion, results of our study suggest that even though random error and technical issues likely are the main sources of the ER variability, results of our study suggest a decrease in ER expression, related with tissue sampling methodology and/or exposure to endocrine therapy.

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**Conflict of interest:** Boris Itkin received funding from Pfizer to assist scientific meetings. Bruno Bustos has an employment position in Pfizer, he is a member of the advisory board in Pfizer and Novartis. The other authors declare no conflict of interest.

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